

REMARKS

Entry of the foregoing, reexamination and reconsideration of the above-identified application are respectfully requested.

Claims 1-43 and 45-80 are pending, however, claims 15-29, 45-51, 59-64 and 74-80 have been withdrawn from further consideration as being directed to a non-elected invention. Claims 1-14, 30-43, 52-58 and 65-73 are being examined in this application.

Claims 35 and 69 have been deleted without prejudice or disclaimer. Claims 1, 11 and 34 have been amended. Claim 1 was amended to recite "isolated." Claims 11 and 34 have been amended to make more clear that the lipid is excreted as "lipid vesicles encapsulating said lipid." This amendment makes claims 11 and 34 consistent with claim 1, in that the added language is similar to that appearing in original claim 1. Claim 58 has been amended to correct the dependency to be from claim 2. No new matter is added by these amendments.

The present Abstract was objected to as being improper. A new Abstract in one paragraph, as requested, is submitted herewith.

Claims 1-3, 7-14, 34-36 and 52-58 have been rejected under 35 U.S.C. §101 as allegedly being directed to nonstatutory subject matter. This rejection has been rendered moot by the instant amendment. Claim 1 has been amended to recite "isolated," as requested. The dependent claims also require the microorganism to be "isolated," since they depend from claim 1 and require each limitation of claim 1.

Withdrawal of this rejection is thus respectfully requested and believed to be in order.

Claims 1-14, 30-43, 52-58 and 65-73 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter not enabled by the specification. This rejection is respectfully traversed.

The claims are said to be directed to a composition obtained by culturing a microorganism, wherein the microorganism is *Mortierella alpina*. That microorganism is said to be essential to the invention and must be "obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public." As stated in the Official Action, a deposit of the microorganism would satisfy this requirement.

Microorganisms which may be employed for practice of the instant invention have been deposited, as set forth on page 10 of the application. An executed Deposit Declaration relating to those deposits is presently being obtained and will be submitted upon receipt.

Applicants further submit that one skilled in the art could practice the claimed invention based upon information provided in the specification and available in the art. As noted on pages 9-10 of the specification, other microorganisms in addition to *Mortierella alpina* could be used. See, page 9, line 28 - page 10, line 9. As noted on page 10, lines 10-16, many of the strains provided in the application are available without limitations from the IFO in Osaka, Japan, the ATCC in the United States, and CBS. The specification also describes how to obtain a microorganism according to the instantly claimed invention. For example, at page 10, line 22 to page 12, line 32. Example 1 at pages 20-22 similarly describes how to obtain a microorganism as claimed. One skilled in the art could thus

practice the claimed invention based upon the information in the specification and publically available resources.

Withdrawal of this rejection is thus respectfully requested and believed to be in order.

Claims 7-9, 31-39, 42-43, 55-57, 59 and 66-73 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. This rejection is respectfully traversed.

The phrase "culture liquid cloudy" in claims 7, 9, 37, 39, 55 and 57 is said to be indefinite because the meaning of the word "cloudy" is unclear. It is respectfully submitted that this phrase would be sufficiently clear to a person skilled in the art. The meaning of "cloudy" as used in the claim would be sufficiently clear to a person skilled in the art, as being used in contrast to the "transparent" medium recited therein. As stated on page 7, lines 30-35 of the application, for example:

Microorganism that accumulate lipids in the cell do not make the medium cloudy during culturing, whereas microorganisms that extracellularly secrete the produced lipids make the medium cloudy, and therefore, microorganisms having an ability of extracellularly secreting lipids can be easily screened by a mere visual confirmation of the degree of cloudiness of the culture liquid.

The phrase "artificially treating" or "artificial treatment" in claims 8, 9, 38, 39, 53, 56, 57 and 70 is said to be unclear and a definition of the phrases is requested. We note that examples of "artificial treatment" are given on page 10. At page 10, line 32 to page 11, line 7, "artificial treatment" is defined as being "mutation, gene manipulation, cell fusion and the like." These phrases would thus be sufficiently clear to a person skilled in the art when read in light of the specification.

The word "directly" in claim 35 is said to be unclear and a definition requested. This objection is now moot in view of the cancellation of claim 35.

Antecedent basis for "arachidonic acid" in claim 58 is requested. This objection is now moot in view of this amendment. Claim 58 was amended to correct an inadvertent error, and to depend from claim 2 rather than claim 12.

In view of the above, withdrawal of the rejection of record is respectfully requested and believed to be in order.

Claims 1-3, 7-9, 12, 30, 32, 33, 53, 56 and 68 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Fukui, as evidenced by Stedman's Medical Dictionary. This rejection is respectfully traversed.

The instant invention relates to a microorganism which (1) "extracellularly secretes an unsaturated fatty acid-containing lipid," and (2) secretes the lipid "as lipid vesicles encapsulating said lipid." *See also, e.g.,* page 7, lines 6-9 and 15-17, of the specification. These are two properties that the claimed microorganism must have, in accordance with claim 1 of the instant invention. The microorganisms of the prior art do not have these two properties. They thus do not disclose a microorganism as claimed. Nor do they suggest such a microorganism having these properties.

In addition, in failing to disclose or suggest such a microorganism as recited in claim 1, the cited prior art also fails to teach a method of using such a microorganism to produce lipid vesicles, unsaturated fatty acids or lipids as recited in dependent claims.

Fukui is discussed in the instant specification. As described on page 4, lines 14-28, Fukui attempted to extracellularly secrete a lipid accumulated in the cell. The

microorganisms of Fukui convert sugar and n-alkanes to lipids and secrete them outside the cell. According to the method of Fukui, triglyceride is directly secreted, *i.e.*, without being encapsulated into a vesicle, outside of the cells. As acknowledged in the instant specification, the Fukui method "has a drawback that the TG that is directly secreted outside of the cell is incorporated into the cell again and metabolized." Page 4, lines 25-28. This drawback is acknowledge in Fukui. For example, page 11, lines 14-20 of the English translation of Fukui states: "On the other hand, the disadvantage of strain L-12 is that once TG is accumulated outside of the cell, it ends up being metabolized after again being incorporated inside the cell. Thus the direction to be taken for breeding a desirable strain lies in inhibiting uptake, promoting discharge and inhibiting metabolism of TG."

The Fukui reference thus does not teach or even suggest the possibility of the lipid containing unsaturated fatty acid being secreted outside of the cells in the form of a lipid vesicle. Secreting the lipid containing unsaturated fatty acid outside of the cells in the form of a lipid vesicle overcomes the disadvantage recognized by Fukui. Lipid encapsulated in lipid vesicles cannot be re-incorporated into the cells and metabolized. Therefore, the fact that triglyceride, which has been secreted outside cells, is re-incorporated into the cells means that the triglyceride is directly secreted without being incorporated into the lipid vesicles, as instantly claimed.

In addition, according to Fukui, *Candida lipolytica* secretes palmitic acid. "Since palmitic acid (sodium salt) precipitates in the medium, it is easy to assess the status of accumulation and production." *See*, page 4, line 2 to the bottom of page 5, line 1, of the English translation. In accordance with this description, the secreted palmitic acid reacts

with sodium ions in the medium to form sodium palmitate, which precipitates. If the secreted palmitate was incorporated into lipid vesicles, in accordance with the instantly claimed invention, the palmitic acid would not react with sodium ions. Therefore, the fact that the secreted palmitic acid precipitates as sodium salt means that the palmitic acid is secreted without being incorporated into vesicles.

Fukui thus does not disclose or suggest a microorganism in accordance with the instantly claimed invention. Nor does Fukui teach a method of using such a microorganism to produce lipids, lipid vesicles or unsaturated fatty acids. Since anticipation requires a description of each element of the claimed invention, Fukui thus fails to anticipate the instantly claimed invention.

Claims 1-6, 14, 34-37, 52, 55 and 69 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Fragrance Journal, as evidenced by Stedman's Medical Dictionary. This rejection is respectfully traversed.

According to Fragrance Journal, Table 2 describes the amount of total lipid per dry cell (*see*, footnote "a" of Table 2). This means that the amount of lipid *in* the cells is described, *not* lipid secreted *outside* the cells. This reference thus fails to describe or even suggest microorganisms which (1) extracellularly secrete unsaturated fatty acids (2) in the form of lipid vesicles incorporating the lipid, as instantly claimed. Nor does the reference teach or suggest methods of using such microorganisms to produce lipids, lipid vesicles or unsaturated fatty acids, as also instantly claimed.

In addition, Table 2 refers to References 35 (Totani et al) and 36 (Yamada et al), a copy of each of which is submitted herewith. At page 328, right column, line 3 to bottom

of page 329, left column, line 8, Totani et al states that "Mycelium and pellicle grown were harvested, dried in a desiccator, and ground in a mortar with pestle using chloroform/methanol 2:1 vol/vol. Lipids were extracted further with the same solvent mixture and interesterified with sodium methoxide. colorless clear methyl ester thus obtained was analyzed ..."

Yamada et al, on page 174, left column, lines 9-5 from the bottom, describes that "the cells were harvested, washed with water and dried in a centrifugal evaporator at 50-60C. Fatty acids in the cells were subjected to methanolysis, extracted with n-hexane and analyzed by gas-liquid chromatography."

In view of these disclosures, it is clear that the fatty acids are not secreted by the microorganisms in Fragrance Journal. Fragrance Journal thus fails to teach a microorganism as instantly claimed, wherein the microorganism (1) extracellularly secrete unsaturated fatty acids (2) in the form of lipid vesicles incorporating the lipid. Since anticipation requires a description of each element of the claimed invention, Fragrance Journal fails to anticipate the claimed invention.

Withdrawal of this rejection is thus respectfully requested. Such action is believed to be in order.

Claims 1-14, 30-43, 52-58 and 65-73 have been rejected under 35 U.S.C. §103(a) as allegedly being obvious over Fukui in view of Fragrance Journal, as evidenced by Stedman's Medical Dictionary. This rejection is respectfully traversed.

As set forth in detail *supra*, neither Fukui nor Fragrance Journal teach or suggest a microorganism as instantly claimed. Neither reference discloses or suggests a

microorganism which (1) extracellularly secrete unsaturated fatty acids (2) in the form of lipid vesicles incorporating the lipid, as instantly claimed. There would have been no motivation to use a *Mortierella alpina* microorganism as instantly claimed, wherein the microorganism (1) extracellularly secrete unsaturated fatty acids (2) in the form of lipid vesicles incorporating the lipid, as instantly claimed. Nor does the reference teach or suggest methods of using such microorganisms to produce lipids, lipid vesicles or unsaturated fatty acids, as also instantly claimed. The combination of references thus similarly fails to render obvious such a microorganism or method of using same, as claimed in the instant application.

Withdrawal of this rejection is respectfully requested and believed to be in order.

Claims 10 and 40 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Fukui in view of Fragrance Journal, as evidenced by Stedman's Medical Dictionary, and further in view of Buxton et al and Harman et al. This rejection is respectfully traversed.

The combination of Fukui and Fragrance Journal fails to teach or suggest the instantly claimed invention, for the reasons set forth *supra*. Each of claims 10 and 40 now requires both (1) that the microorganism extracellularly secrete a lipid, and (2) that the lipid is secreted as a lipid vesicle encapsulating the lipid. Claim 10 depends from claim 1, and claim 40 depends from claim 34. Claim 34 was amended to be consistent with claim 1 and recite both that the microorganism extracellularly secrete a lipid and that the lipid is secreted as a lipid vesicle encapsulating the lipid.

As stated *supra*, neither Fukui nor Fragrance Journal discloses or suggests such a microorganism which (1) extracellularly secretes a lipid (2) as a lipid vesicle encapsulating the lipid. Nor do the additionally cited references of Buxton et al and Harman et al disclose or suggest such a microorganism as claimed. The combination of references thus fails to disclose or even suggest the invention as recited in claims 10 and 40.

Withdrawal of this rejection is respectfully requested and believed to be in order.

Claim 11 has been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Higashiyama et al. This rejection is respectfully traversed.

Claim 11 has been amended to recite that the lipid is extracellularly secreted "as lipid vesicles encapsulating said lipid," in accordance with the language in claim 1. In view of this amendment, Higashiyama et al fails to disclose or suggest the claimed invention. Higashiyama et al does not teach or suggest a filamentous fungus "having a property of extracellularly secreting a lipid as lipid vesicles encapsulating said lipid, wherein said lipid contains unsaturated fatty acids." Higashiyama et al neither discloses nor suggests a fungus which can extracellularly secrete a lipid as a lipid vesicle encapsulating the lipid.

Higashiyama et al thus fails to anticipate the instantly claimed invention, since each element of the claim must be disclosed for anticipation.

Withdrawal of this rejection is thus respectfully requested. Such action is believed to be in order.

Applicants further note that the instantly claimed microorganisms are beneficial to the art. As disclosed in the specification, such a microorganism is advantageous in the art. The advantages are as follows:

(a) The lipid vesicles have excellent dispersion properties to water and hydrophilic substances, and therefore, they can be added to foods containing no fat or oil, the addition to which having conventionally been impossible.

(b) The lipid can stably retain the lipids to prevent air oxidation.


(c) Since lipid is encapsulated in the lipid vesicles, once the lipid is secreted, the lipid cannot be reincorporated into the cells and cannot be metabolized in the cells. Therefore, the lipid encapsulated in the lipid vesicles is efficiently accumulated in the culture medium, and can be easily recovered.

In view of the above, all of the pending claims are thus respectfully believed to be in condition for allowance. It is respectfully submitted that all rejections have been overcome by the above amendments and/or arguments. Thus, a Notice of Allowance is respectfully requested.

In the event that there are any questions relating to this amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (650) 622-2360 so that prosecution of the application may be expedited.

Respectfully submitted,

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Attachment to Reply and Amendment

Marked-up Claims 1, 11, 34 and 58

1. (Amended) [A] An isolated microorganism which extracellularly secretes an unsaturated fatty acid-containing lipid as lipid vesicles encapsulating said lipid.

11. (Amended) A filamentous fungus having a property of extracellularly secreting a lipid as lipid vesicles encapsulating said lipid, wherein said lipid contains [containing] unsaturated fatty acids.

34. (Amended) [A] An isolated microorganism having a property of extracellularly secreting a lipid as lipid vesicles encapsulating said lipid, wherein said lipid contains [containing] unsaturated fatty acids that have 18 carbons and three or more double bonds or 20 or more carbons and two or more double bonds.

58. (Amended) The microorganism according to claim [12] 2, wherein said unsaturated fatty acids are arachidonic acid.